

What is claimed is:

- 5 *Sub 1*
1. A vector system for selectively packaging a replication defective adenovirus nucleic acid sequence in an adenovirus capsid based on adenovirus serotype, comprising:
- a first replication defective adenovirus sequence comprising a first adenovirus serotype *cis*-acting packaging sequence and a heterologous nucleic acid;
- a second replication defective adenovirus sequence comprising a second adenovirus serotype *cis*-acting packaging sequence, lacking the ability to produce a polypeptide having the activity of a second adenovirus serotype 52/55 kDa *trans*-acting protein; and
- a nucleic acid sequence encoding a polypeptide having the activity of a first adenovirus serotype 52/55 kDa *trans*-acting protein and lacking the activity of a second adenovirus serotype 52/55 kDa *trans*-acting protein.
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- 15 2. The vector system of claim 1, wherein the adenovirus capsid, packaging and 52/55 kDa *trans*-acting protein encoding sequences are human adenovirus sequences.
- 20 *Sub 2*
3. The vector system of claim 2, wherein the first and second adenovirus serotypes are adenovirus type 2 (Ad2), adenovirus type 5 (Ad5), adenovirus type 7 (Ad7), adenovirus type 12 (Ad12), adenovirus type 17 (Ad17), or adenovirus type 40 (Ad40), and the first serotype differs from the second serotype.
- 25 4. The vector system of claim 2, wherein the first adenovirus serotype is adenovirus type 5 and the second adenovirus serotype is adenovirus type 7.
5. The vector system of claim 2, wherein the first adenovirus serotype is adenovirus type 7 and the second adenovirus serotype is adenovirus type 5.
- 30 *Sub 3*
6. The vector system of claim 1, wherein the first replication defective adenovirus sequence cannot produce a complete adenovirus capsid.

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7. The vector system of claim 6, wherein the first replication defective adenovirus sequence is encapsidated in a capsid comprising at least one polypeptide encoded by the second replication defective adenovirus sequence.
 8. The vector system of claim 6, wherein the first replication defective adenovirus sequence is encapsidated in a capsid encoded by the second replication defective adenovirus sequence.
 9. The vector system of claim 1, wherein the replication defective adenovirus comprises a defective or modified adenovirus E1 gene, E2A gene, E2B gene, E3 gene, E4 gene, E4 promoter, penton gene, fiber gene or hexon polypeptide gene or combination thereof.
 10. The vector system of claim 1, wherein the inability to produce a functional 52/55 kDa *trans*-acting protein is due to a mutation in the sequence encoding the protein.
 11. The vector system of claim 10, wherein the mutation is a missense mutation, a point mutation, a frameshift mutation or a deletion mutation.
 12. The vector system of claim 1, wherein the second replication defective adenovirus sequence further comprises the nucleic acid sequence encoding the polypeptide having the activity of the first serotype 52/55 kDa *trans*-acting protein.
 13. The vector system of claim 1, wherein the nucleic acid sequence encoding the polypeptide having the activity of the first serotype 52/55 kDa *trans*-acting protein further comprises an adenovirus replication competent host cell.
 14. The vector system of claim 13, wherein adenovirus replication competent host cell is a 293 cell line.
 15. The vector system of claim 1, wherein the polypeptide having the activity of a first serotype 52/55 kDa *trans*-acting protein is a first serotype 52/55 kDa *trans*-acting protein.

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16. The vector system of claim 1, wherein the first replication defective adenovirus sequence lacks at least one nucleic acid sequence needed to produce a capsid and further comprises a nucleic acid sequence encoding a polypeptide having the activity of a first adenovirus serotype 52/55 kDa *trans*-acting protein.

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17. A vector system for selectively packaging a replication defective adenovirus nucleic acid sequence in an adenovirus capsid based on adenovirus serotype, comprising: a first replication defective adenovirus sequence comprising a first adenovirus serotype *cis*-acting packaging sequence and a heterologous nucleic acid; and, a second replication defective adenovirus sequence comprising a second adenovirus serotype *cis*-acting packaging sequence, a nucleic acid sequence encoding a polypeptide having the activity of a first adenovirus serotype 52/55 kDa *trans*-acting protein, lacking the ability to produce a polypeptide having the activity of a second adenovirus serotype 52/55 kDa *trans*-acting protein.

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18. A vector system for selectively packaging a replication defective adenovirus nucleic acid sequence in an adenovirus capsid based on adenovirus serotype, comprising: a first replication defective adenovirus sequence comprising a first adenovirus serotype *cis*-acting packaging sequence and a heterologous nucleic acid; a second replication defective adenovirus sequence comprising a second adenovirus serotype *cis*-acting packaging sequence, lacking the ability to produce a polypeptide having the activity of a second adenovirus serotype 52/55 kDa *trans*-acting protein; and, a cell comprising a nucleic acid sequence encoding a polypeptide having the activity of a first adenovirus serotype 52/55 kDa *trans*-acting protein.

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19. A vector system for selectively packaging a replication defective adenovirus nucleic acid sequence in an adenovirus capsid based on adenovirus serotype, comprising: a first replication defective adenovirus sequence comprising a first adenovirus serotype *cis*-acting packaging sequence and a heterologous nucleic acid; a second replication defective adenovirus sequence comprising a second adenovirus serotype *cis*-acting packaging sequence, lacking the ability to produce a polypeptide having the activity of a second adenovirus serotype 52/55 kDa *trans*-acting protein; and, an expression cassette comprising a

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nucleic acid sequence encoding a polypeptide having the activity of a first adenovirus serotype 52/55 kDa *trans*-acting protein.

5 20. A vector comprising a replication defective adenovirus sequence comprising a first adenovirus serotype *cis*-acting packaging sequence, a nucleic acid sequence encoding a functional second adenovirus serotype 52/55 kDa *trans*-acting protein, wherein the second adenovirus serotype 52/55 kDa *trans*-acting protein does not have the activity of a first adenovirus serotype 52/55 kDa *trans*-acting protein, lacking the ability to produce a polypeptide having the activity of a first adenovirus serotype 52/55 kDa *trans*-acting protein.

10 21. The vector of claim 20, further comprising at least one adenoviral nucleic acid sequence needed to produce an adenoviral capsid.

15 22. The vector of claim 21, further comprising sufficient adenoviral nucleic acid sequence to produce a complete adenoviral capsid when the vector is expressed in an adenovirus replication-competent host cell.

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23. The vector of claim 20, wherein the first and second adenovirus serotypes are adenovirus type 2 (Ad2), adenovirus type 5 (Ad5), adenovirus type 7 (Ad7), adenovirus type 12 (Ad12), adenovirus type 17 (Ad17), or adenovirus type 40 (Ad40), and the first serotype differs from the second serotype.

25 24. The vector of claim 23, wherein the first adenovirus serotype is adenovirus type 5 and the second adenovirus serotype is adenovirus type 7.

25 25. The vector of claim 23, wherein the first adenovirus serotype is adenovirus type 7 and the second adenovirus serotype is adenovirus type 5.

30 26. A transformed or isolated infected cell comprising the vector system of claim 1 or the vector of claim 20.

27. A kit for making adenovirus encapsidated replication defective sequences comprising
a first adenovirus serotype *cis*-acting packaging sequence and a heterologous
nucleic acid,

a second replication defective adenovirus sequence comprising a second
adenovirus serotype *cis*-acting packaging sequence, lacking the ability to produce a
polypeptide having the activity of a second adenovirus serotype 52/55 kDa *trans*-acting
protein, and

a nucleic acid sequence encoding a polypeptide having the activity of a first
adenovirus serotype 52/55 kDa *trans*-acting protein.

28. The kit of claim 27, wherein the nucleic acid sequence encoding a polypeptide having
the activity of a first adenovirus serotype 52/55 kDa *trans*-acting protein further comprises an
adenovirus replication competent cell.

29. The kit of claim 27, wherein the nucleic acid sequence encoding a polypeptide having
the activity of a first adenovirus serotype 52/55 kDa *trans*-acting protein further comprises an
expression cassette.

30. The kit of claim 27, wherein the second replication defective adenovirus sequence
further comprises the nucleic acid sequence encoding a polypeptide having the activity of a
first adenovirus serotype 52/55 kDa *trans*-acting protein.

31. A method of producing a replication defective encapsidated adenovirus gene transfer
vector, comprising the following steps:

(a) transforming or infecting into adenovirus replication competent host cells

(i) a first replication defective adenovirus sequence comprising a first
adenovirus serotype *cis*-acting packaging sequence and a heterologous gene,

(ii) a second replication defective adenovirus sequence comprising a
second adenovirus serotype *cis*-acting packaging sequence, lacking the ability to
produce a polypeptide having the activity of a second adenovirus serotype 52/55 kDa
trans-acting protein, and

(iii) a nucleic acid sequence encoding a polypeptide having the activity of a first adenovirus serotype 52/55 kDa *trans*-acting protein; and

(b) culturing the cells under conditions where the first replication defective adenovirus sequence is encapsidated to produce a replication defective adenovirus gene transfer vector.

32. A method of producing a replication defective encapsidated adenovirus gene transfer vector, comprising the following steps:

(a) transforming or infecting into an adenovirus replication competent host cell two adenovirus replication defective sequences, wherein the cell comprises a nucleic acid sequence encoding a polypeptide having the activity of a first adenovirus serotype 52/55 kDa *trans*-acting protein,

(i) a first replication defective adenovirus sequence comprising a first adenovirus serotype *cis*-acting packaging sequence and a heterologous gene, and

(ii) a second replication defective adenovirus sequence comprising a second adenovirus serotype *cis*-acting packaging sequence, lacking the ability to produce a polypeptide having the activity of a second adenovirus serotype 52/55 kDa *trans*-acting protein; and

(b) culturing the cells under conditions where the first replication defective adenovirus sequence is encapsidated to produce a replication defective adenovirus gene transfer vector.

33. A method of producing a replication defective encapsidated adenovirus gene transfer vector, comprising the following steps:

(a) transforming or infecting into an adenovirus replication competent host cell two adenovirus replication defective sequences

(i) a first replication defective adenovirus sequence comprising a first adenovirus serotype *cis*-acting packaging sequence, a heterologous gene and a nucleic acid sequence encoding a polypeptide having the activity of a first adenovirus serotype 52/55 kDa *trans*-acting protein, and

(ii) a second replication defective adenovirus sequence comprising a second adenovirus serotype *cis*-acting packaging sequence, lacking the ability to

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5 produce a polypeptide having the activity of a second adenovirus serotype 52/55 kDa *trans*-acting protein; and

(b) culturing the cells under conditions where the first replication defective adenovirus sequence is encapsidated to produce a replication defective adenovirus gene transfer vector.

10 34. The method of claim 31, 32, or 33 wherein the second replication defective adenovirus sequence further comprises sufficient adenoviral nucleic acid sequence to encode a complete adenoviral viral capsid.

15 35. A vector for selectively packaging replication defective nucleic acid sequences in adenovirus capsids based on adenovirus serotype, comprising a replication defective adenovirus sequence comprising an adenovirus serotype 7 (Ad7) *cis*-acting packaging sequence, a nucleic acid sequence encoding a polypeptide having the activity of an adenovirus serotype 5 (Ad5) 52/55 kDa *trans*-acting protein, and sufficient adenoviral nucleic acid sequence to encode a viral capsid, lacking the ability to produce a polypeptide having the activity of an adenovirus 7 serotype 52/55 kDa *trans*-acting protein.

20 36. A pharmaceutical composition comprising an encapsidated replication defective adenovirus, made using the vector system of claim 1, substantially free of helper virus, and a pharmaceutically acceptable excipient.

25 37. The pharmaceutical composition of claim 36, wherein the pharmaceutical composition is 99% free of helper virus.

38. A method of delivering a heterologous nucleic acid to a cell comprising transforming or infecting a cell with the pharmaceutical composition of claim 36.

30 39. The method of claim 38, wherein the pharmaceutical composition is administered to a patient systemically, regionally or locally.

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